*Vol. V.* *September, 1903.* *No. 4*

# BIOLOGICAL BULLETIN.

## THE SPERMATOGENESIS OF THE MYRIAPODS.—II. ON THE CHROMATIN IN THE SPERMATOCYTES OF SCOLOPENDRA HEROS.

MAULSBY W. BLACKMAN.

In a detailed study of the spermatocyte changes in *Scolopendra heros*, now practically ready for publication, the multiplicity of subjects requiring consideration is such that it is deemed advisable to prepare a series of shorter papers, in each of which some particular class of structures may be considered to the practical exclusion of the others. It is hoped that in this manner the confusion which necessarily occurs where the whole subject is treated at one time may be avoided. In this, the first of the series of articles, the chromatin structures alone will be treated.

The spermatogonia of *Scolopendra* are small cells of an elongated, irregular shape lying parallel to the long axis of the follicle, and containing an oval nucleus (Fig. 1). During the resting stages the chromatin is all aggregated into one rather large, spherical nucleolus-like body, usually situated at the periphery of the nucleus and apposed to its membrane. The remainder of the nuclear space is filled by an irregular network of granular fibers apparently differing in no way from the cytoplasmic network without the nucleus. In staining reaction the nucleolar body mentioned conforms in all respects to a chromatin body as it indubitably is. When stained with Heidenhain's iron-hæmatoxylin, this structure retains the coloring matter after all other morphological elements of the cell have become almost colorless. In lightly stained preparations evidences appear which warrant the assertion that the body in question is not strictly homogeneous in structure, but probably includes in its composition linin as well as chromatin. With Flemming's three-color method the "nucleolus" takes the dense red stain characteristic of closely

aggregated chromatin; and with the Ehrlich-Biondi mixture, following the action of suitable fixatives, assumes the green color usual to chromatin treated by this reagent. Numerous other stains of a greater or less value as micro-chemical tests were used and with all these the chromatin nature of this body was invariably demonstrated.

The character of this nucleolar body which, for reasons late made apparent, I shall call the *karyosphere* is still further indicated by its behavior in the prophase of the spermatogonium. Owing to the advanced development of my material I have been unable to study any but the last generations of these cells, but I believe that the phenomena here observed are common to all generations of the secondary spermatogonia. In all cases studied, the active prophase is characterized by the presence within the nuclear vesicle of 33 small aggregations of chromatin and the complete absence of the karyosphere (Fig. 2), thus giving a logical basis to the conclusion that the chromosomes are derived directly from the substances of the karyosphere. Of these 33 chromosomes 32 are characterized in the earlier prophases by their granular consistency, while the remaining one is plainly distinguishable on account of its homogeneous nature and its clear-cut outline. This modified chromatic element is the accessory chromosome, first recognized as a specialized chromosome by McClung, '99, and later found to be probably of universal distribution in the male cells of arthropods.

It will be noted that the number of chromosomes, 33, given above as characteristic of the spermatogonium is not a multiple of two as is generally considered to be necessarily the case of immature germ cells. The reason for this fact has to do with the peculiar character of the accessory chromosome, and can readily be explained when the later behavior of this element is known.

During the following phases in the mitosis of the last generation of secondary spermatogonia, nothing of especial interest with regard to the chromatin occurs until the telophase is reached. This phase endures for a considerable time as is shown by the great number of slightly different stages present and by the fact that more spermatogonia are found in this condition than in any

other stage of mitosis. In the early telophase where the two new cells are almost completely constricted the chromatin is arranged in a densely packed mass of chromosomes in which the individual elements are indistinguishable. Later (Fig. 3) these elements

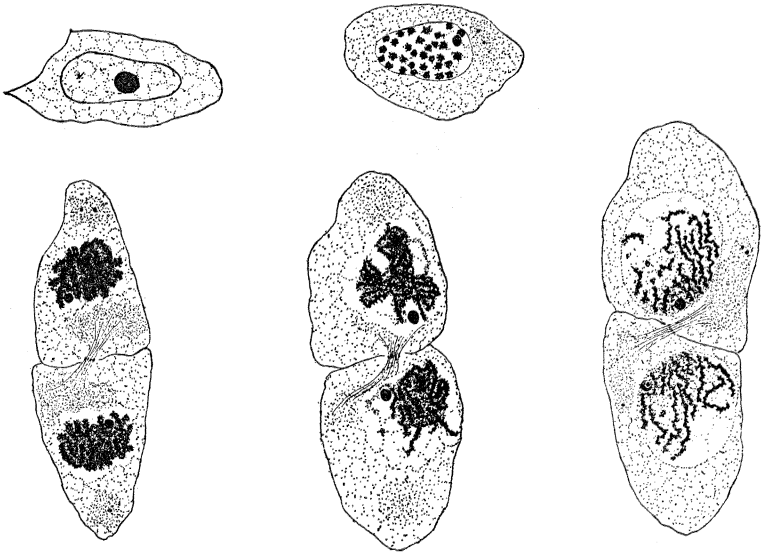


FIG. 1. × 1,440 dia. Spermatogonium of *Scolopendra heros* in the condition of rest. All of the chromatin is aggregated into one mass, the karyosphere.

FIG. 2. × 1,440 dia. Spermatogonium in prophase. The chromatin is all withdrawn from the karyosphere and is now in the form of 33 small chromosomes all of which, with the exception of the accessory chromosome, are of a granular consistency. This element is homogeneous. The centrosomes are to be seen in the cytoplasm near the nucleus.

FIG. 3. × 1,440 dia. Telophase of the last spermatogonium. Synapsis. Cytoplasmic division nearly complete. All chromosomes with exception of accessory, becoming granular. No nuclear membrane. Centrosomes at poles of the cell.

FIG. 4. × 1,440 dia. Later telophase. Synapsis. Chromosomes have lengthened still more. Accessory chromosome still intact. Growth of the cell has begun.

FIG. 5. × 1,440 dia. Early spermatocyte. Nuclear membrane beginning to form. Accessory has taken up a peripheral position. Mass of chromatin has loosened considerably and is now seen to consist of segments equal in number to one half the spermatogonial elements, minus the accessory chromosome. Centrosomes have migrated from their polar position.

begin to lose their homogeneous consistency and to lengthen out into densely granular segments. Owing to the dense massing of the chromosomes during this and following stages, the ex-

act nature of the changes taking place cannot be learned. Several facts are however very apparent. Of these one of the most important is this :—At the time when all the other morphological constituents of the mass of chromatin are undergoing very fundamental changes, one of these elements remains unaltered. While all of the neighboring chromosomes lose their definite outlines and are changed into elongated threads of a granular structure one, the accessory chromosome, does not participate in this metamorphosis but apparently retains all of the properties characteristic of it during metakinesis. While this difference in consistency is the most apparent discrepancy existing between the accessory chromosome and the ordinary chromatic elements, as we shall see presently, it is by no means the most important one.

As the telophase advances the chromosomes continue to lengthen out into long threads. At first, as we have seen, these filaments form a dense mass which is surrounded by no membrane marking off the nuclear area from the cytosome. As the chromosomes become more diffuse this mass also becomes less dense and the individual segments are not so closely apposed to each other. This stage is shown in Fig. 4, where the chromatin of each of the two daughter cells is in the form of an irregular, more or less closely knotted, mass of granular filaments. This mass is contained in a large clear vacuole having no visible network of linin or cytoplasm and bounded by no definite membrane. The appearance of the chromatin grouped in a diffuse mass upon one side of this vesicle suggests very strongly a comparison between this stage in *Scolopendra* and the "synapsis" in elasmobranchs, as described by Moore, '95, and later in different objects by numerous other authors. In all the reported cases with which I am acquainted, however, this massing of the chromatin upon one side of the nuclear vesicle occurs at a considerably later stage than the early or mid-telophase. Paulmier '98, and Montgomery '98, both figure it as taking place after the formation of the chromatic spireme. McClung, '00, denies the normal existence of any such massing of the chromatin in the *Acrididæ*, referring such appearances to the distorting effects of the fixing reagents employed. By the majority of investigators upon male cells this massing of the chromatin is used

as the criterion of the synapsis or pseudo-reduction, but Montgomery, '01, apparently abandoning his former views upon the subject, asserts, probably with very good reason, that in reality synapsis occurs at a considerably earlier stage. In *Peripatus*, '00, he is able to study the manner of this union of the chromosomes and from observations seems to have good grounds for the assertion that synapsis is accomplished by an end to end union, in pairs, of entire chromosomes during the retrogressive stages of the telophase of the last spermatogonial division.[1]

In *Scolopendra*, owing to the small size of the spermatogonia and the extreme minuteness of the spermatogonial chromosomes, as well as their larger number and close aggregation during the telophase, the manner of union and the details of the process cannot be studied; but it can be stated with the greatest certainty that pseudo-reduction occurs during the telophase of the last spermatogonium, and is completed before the reconstruction of the nuclear membrane. At the time of the formation of this structure, the nuclear space is occupied by sixteen elongated segments of chromatin and resembles very closely the nucleus in insect cells with the exception that the nuclear area is much larger in proportion to the amount of chromatin and thus the segmented character of the chromatin is evident (Figs. 5 and 6). Besides these sixteen diffuse segments of chromatin, the accessory chromosome is also plainly visible within the nucleus. It still preserves its distinctive characteristics and has changed very little from its condition during the preceding division. To be sure, it has increased in size as have all parts of the cell, but this increase may all be referred to natural growth. This element takes no part whatever in the process of synapsis. During the spermatogonial stages it is a simple chromatic structure and in the following spermatocyte period it still retains its univalent character when all of the other chromosomes are bivalent.

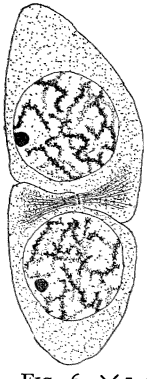
The completion of cell division and the union of the chromosomes occurring during the telophase have occupied considerable time, as is shown by several facts. Cells in various stages of the

[1] A late paper by W. S. Sutton upon "The Morphology of the Chromosome Group in *Brachystola magna*" contains further and much more convincing proof of the truth of this process. Mr. Sutton is able to trace plainly the union of the chromosomes and to show that it is undoubtedly an end-to-end union of entire elements.

telophase are more numerous in the material examined than those in any other condition of the spermatogonium. A large number of different stages may be distinguished. The cells in the early telophase are small, while those in which the nuclear wall is reconstructed are considerably larger, showing that already the growth period has begun. (Compare Figs. 3 and 5.)

With the completion of the nuclear membrane after the last spermatogonial mitosis, the cells no longer belong to the first division of the spermatogenetic cycle, but now contain the matured number of chromosomes and are spermatocytes. In insect material the transformation is not completed until a period apparently considerably later. However, I believe this difference is merely in appearance, lying in the fact that the nuclear membrane is reconstructed much earlier in insect cells.

At this stage the cells of *Scolopendra* enter upon a period remarkable for the extraordinary changes which take place in their



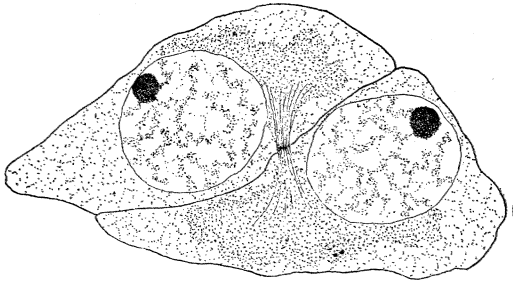


FIG. 6. × 1,440 dia. Slightly later stage. The chromatin segments scattered throughout entire nuclear space.

FIG. 7. × 1,440 dia. Chromatin partly gathered about the accessory chromosome to form the karyosphere. Remaining chromatin of the cell present in the form of very diffuse segments. Spindle remains of last spermatogonial divisions still persist.

structure. At first glance the most striking of these changes seems to be the enormous increase in the size of the cells (Figs. 6, 7 and 8). This growth I have already described briefly in a preliminary paper and shall have occasion to describe more in detail in subsequent communications. In this connection it will suffice to say that very often the diameter of the larger sperma-

tocytes to that of the spermatogonium stands in a ratio of ten to one.

Striking as this great increase in the size of the cells certainly is, it is not as remarkable as are the changes which occur in the cell in general and especially in the nucleus. Shortly after the formation of the nuclear membrane, the chromatin segments leave the tangled mass at one side of the nucleus (Fig. 5), and arrange themselves irregularly throughout the nuclear space (Fig. 6). At the same time they shorten and thicken and, as the nucleus is now quite large, the individual elements may readily be distinguished and their number counted. In all favorable cases

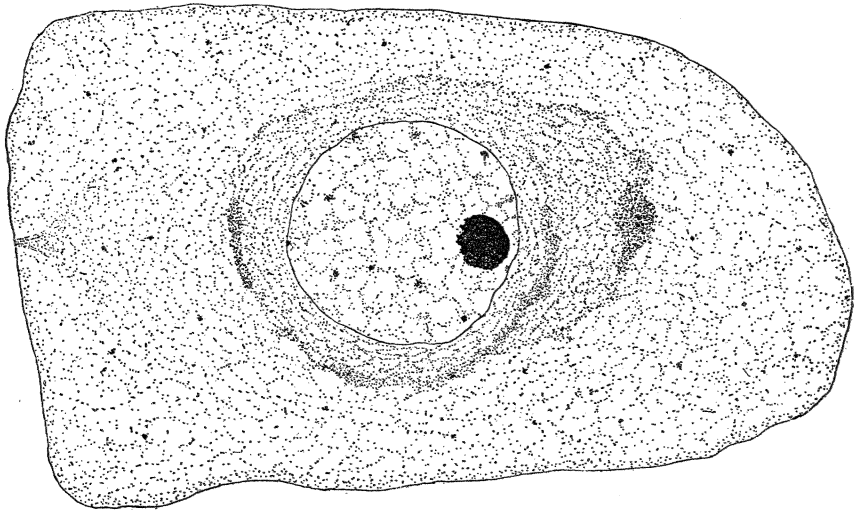


FIG. 8. × 1,440 dia. Pseudo-germinal vesicle stage of the spermatocyte of *Scolopendra heros*. Chromatin all aggregated in karyosphere which here plainly shows except at one point a spongy or reticular structure. This dense portion undoubtedly represents the accessory chromosome. Persisting spindle still visible. Centrosomes to be seen imbedded in the zone of archoplasm surrounding the nucleus.

in which this count has been taken it has been found that there are seventeen chromosomes present (sixteen granular segments and the accessory chromosome), the number later found in the metaphase. At this time (Fig. 6) the cells resemble insect spermatocytes more closely than at any other stage. They are now in a condition apparently comparable in all particulars to that of the ordinary sperm cell in the "segmented spireme"

stage. This is true both with regard to the history of the cell and as regards the morphology of its various structural elements. But from now on the behavior in *Scolopendra* differs very markedly from that of corresponding cells in other animals. In other arthropods at this stage growth is practically completed and the maturation mitoses immediately ensue. In *Scolopendra* the subsequent processes are very different. The growth period has hardly begun and the maturation divisions do not occur until considerably later (probably several weeks or even months). In insects the segmented spireme is considered one of the earlier stages of the active prophase, while in chilopods a condition more closely approaching a true rest stage than that occurring at any other time in the history of the spermatocytes, intervenes between this stage and the first maturation mitosis.

During this intervening stage the history of the spermatocytes parallels in nearly all respects that of the typical female germ cell of a like generation, and the changes which take place result in a structure which if isolated would certainly be mistaken for an immature egg.

As I have reported in a preliminary paper, this resemblance is true not only of the cytoplasmic but of the nuclear elements as well. As the cell continues in its growth the chromatin segments become larger and more diffuse. They no longer retain the stains with the persistency which has characterized them heretofore. This is probably due entirely to the fact that the granules are farther apart and not to a change in the chemical nature of the chromatin. Gradually they break down and their subtance is accumulated about the accessory chromosome, thus seemingly increasing the bulk of this element greatly (Fig. 7). This process continues until finally all of the chromatin of the cell is aggregated in one large intensely staining body situated peripherally in close contact with the nuclear membrane (Fig. 8). The remainder of the nucleus is occupied by a beautiful regular reticulum, the achromatic character of which is shown by the fact that it stains even less densely than the cytoplasmic reticulum immediately without the nucleus.

In a preliminary paper upon *Scolopendra* spermatocytes I stated that I believed this nucleolus-like body to be a homo-

geneous mass of chromatin. Since then, however, I have studied this structure under more favorable circumstances, and am able to demonstrate that this is not true. In my earlier studies sections six and two thirds micra thick were used and these were studied under a magnification of one thousand diameters. In arriving at my later results thin sections two to three micra thick were used as well as the thicker ones. These were stained in varying intensities with Heidenhain's iron-hæmatoxylin and were studied at a magnification of twelve hundred to eighteen hundred diameters. With these improved conditions it is found that this body, which I shall hereafter call the *karyosphere*, is by no means a simple homogeneous sphere of chromatin, but on the contrary is a rather complex structure consisting of chromatin, linin and

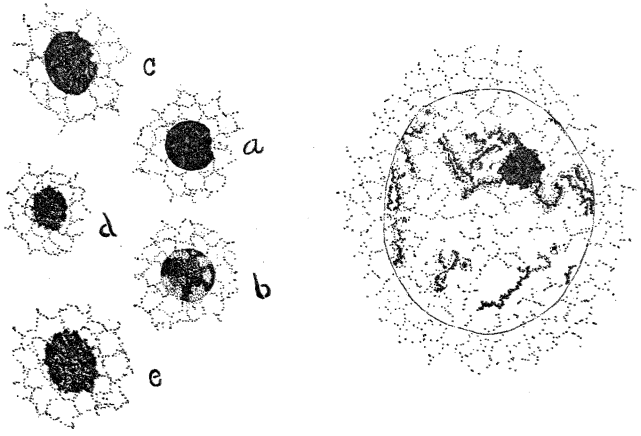



FIG. 9. × 1,440 dia. Karyosphere as seen in various preparations; *a*, as it appears in thick densely stained sections; *b*, karyosphere in which the chromatin segments are massed together by the action of the fixing reagents; *c*, thin lightly stained section of karyosphere showing the real normal structure; *d*, section through one side of karyosphere; *e*, karyosphere in early prophase shortly before the appearance of the chromosomes.

FIG. 10. × 1,440 dia. Nucleus of first spermatocyte in prophase, showing the origin of the chromosomes from the karyosphere. A number of segments have already become detached and lie free in the nucleus while others are still connected with the karyosphere. Those detached have already segmented longitudinally.

karyolymph. It is a mass of fine granular filaments of chromatin so closely gathered about the accessory chromosome as to present, under ordinary conditions and amplification, the appearance of an irregular homogeneous sphere of pure chromatin (Fig. 9, *a*).

Upon higher magnification, sections of this karyosphere usually present a granular or spongy appearance as shown in Fig. 9, *c*. In other cases the chromatin is more or less collected into certain areas forming a coarse cluster in the center from which processes extend toward the periphery (Fig. 9, *b*). Here the body still retains its approximately spherical form, the portion between the processes not staining with the chromatin stains but showing the plasma reaction. Quite often, also, we find a karyosphere which presents the appearance shown in Fig. 9, *c*. This I regard as the typical form. It consists of very fine and closely aggregated mass of chromatin filaments arranged in the form of a more or less perfect sphere. Upon one side of this mass when the section is cut through the right plane is a smaller homogeneous body, the accessory chromosome (Fig. 9, *d*, *e*). The remainder of the karyosphere is made up of irregularly arranged chromatic strands between which minute interstices, undoubtedly filled with karyolymph, may be discovered by careful focusing.

Thus it will be seen that during the pseudo-germinal vesicle stage,[1] the karyosphere, with the exception of a membrane, possesses all of the essential elements of a nucleus — chromatin, linin (upon which the chromatin is arranged) and karyolymph. It is in fact a "nucleus within a nucleus" similar to that described by Carnoy in the closely allied genera of chilopods, *Lithobius*, *Scutigera* and *Geophilus*. This structure which he calls the "nucleole noyau," behaves similarly in all essential respects during the first spermatocyte to the karyosphere in *Scolopendra heros*.[2] It is derived from the chromatin of the nucleus in a similar manner and during the first maturation mitosis behaves in a way essentially alike in all respects.

Carnoy by no means stands alone in the assertion that functional chromatin may and does assume the form of nucleolus-like bodies during resting periods between mitoses, although the structures found by him in *Lithobius*, *Scutigera*, etc., are more highly organized than those reported by others. Among those who have observed that the "chromatin nucleolus" is derived

[1] See former paper.

[2] Carnoy failed to find a "nucleole noyau" in *S. dalmatica*. He considers the intranuclear body in the cells of this animal a true plasmasome in no way related to the structure found in *Lithobius* and other chilopods.

from the chromatin reticulum may be mentioned the following: Blochmann, '82 (*Neritina*); Van Beneden, '83 (*Ascaris*); Van Bambeke, '85 (general); Carnoy, '85 (*Arthropoda*); Rabl, '85 (*Salamandra*); O. Schultze, '87 (*Rana* and *Triton*); Davidhoff, '89 (*Distaplia*); Hermann, '89 (*Mus*); McCallum, 91 (*Echinodermata*); Fick, '93 (*Axolotl*); Holl, '93 (*Mus*); Jordan, '93 (*Newt*); Mertens, '94 (*Pica*); Metzner, '94 (*Salamandra*); McCallum, '95 (*Necturus*, also in plants); Sobotta, '95 (*Mus*); R. Hertwig, '96 (poisoned eggs of *Echinodermata*); Carnoy and Lebrun, '97, '98, '99, '00 (*Amphibia*); Eisen, '00 (*Batrachoseps*); Wilson, '01 (chemically fertilized eggs of *Toxopneustes*); and Blackman, '01 (*Scolopendra*). In many of these animals the process has been followed in such detail that no reasonable doubt can exist as to the accuracy of the results obtained. In other cases the conclusions are not so well supported. In several instances all of the chromatin is not withdrawn from the nuclear reticulum. This is especially true of the cells of Amphibia (McCallum, Jordan, Fick, Eisen, *et al.*). In other batrachian cells all of the chromatin is at certain stages collected in a number of granular masses which also contain linin (O. Schultze, Carnoy and Lebrun, *et al.*). In *Mus*, Hermann finds that at first there are several bodies in the spermatid nucleus but these later fuse to form a single large karyosphere. In this he is confirmed by Sobotta.

Other authors state that all of the chromatin of the cell is withdrawn from the nuclear network and deposited in one large "chromatin nucleolus." Such appearances have been observed and carefully studied by Blochmann, Carnoy, Davidhoff, Hermann, Holl, Sobotta, R. Hertwig, Wilson and others. That the results of such well-known investigators should be descredited or received with scepticism seems strange, yet the majority of cytologists seem not to believe that chromatin may normally be massed in a nucleolus-like body and later act as the functional chromatin of the cell.

Now let us inquire whether such scepticism is justifiable? If it can be shown that in the Protozoa such aggregates of chromatin are of common occurrence normally, certainly it is allowable to conclude that at least some metazoan cells should retain this characteristic. With regard to the intranuclear structures of

Protozoa, Calkins has this to say: "A distinct plasmosome or true nucleolus comparable to the analogous structure in Metazoa apparently exists in no case save possibly in *Actinosphærium*, and even here is limited to a passing phase during mitosis (Hertwig, '98). It is probable that the structures which have been almost invariably but erroneously called nucleoli do not belong at all to this category of nuclear elements but represent either the functional chromatin which is aggregated into a central mass (karyosome) during the quiescent or vegetative period of cell life, or the intra-nuclear division center." From the work of Grüber ('83), Rhumbler ('93), Labbe (96), Hertwig (98), Calkins ('98, '01), and others, we must conclude that chromatin bodies resembling nucleoli more or less closely are of very frequent occurrence in unicellular animals. From Calkins' ('01) review of these investigations it is evident that in its primitive condition the chromatin is present in Protozoa in the form of dense homogeneous masses of chromatin (karyosomes) which act as the *nuclei* of these undifferentiated cells. In higher types the nuclei are more complicated. The chromatin may still occur in simple masses, but these are contained within a nuclear membrane which also encloses material other than chromatin (karyoplasm and karyolymph). The spireme condition so characteristic of the chromatin of metazoan germ cells is not commonly found in Protozoa and when present, exists for only a short time.

The karyosomes found in some of the higher types of protozoan nuclei (*Actinosphærium*, Hertwig) are not homogeneous bodies of chromatin, but, besides this substance, also contain linin. This linin often forms a reticulum upon which the chromatin is deposited in the form of granules, an arrangement very similar to that found in the nuclei of metozoan cells, and gives rise to a structure which is similar to the chromatin reticulum of the more differentiated nucleus. It is, however, still more strikingly like the spireme structure of the karyosphere in appearance. That it is different in some respects, however, is shown by comparing the subsequent behavior of the two structures. The differences are what would be expected when we take into consideration the fact that one is contained in a protozoan cell while the other is in a metazoan cell. The chromatin elements are much more firmly

established in the higher animals and hence it is to be expected that when the karyosphere breaks down, the resulting fragments should be distinct chromosomes. In protozoa the conditions are different. The chromosomes are not such definite structures and hence when the karyosphere of *Actinosphærium* disintegrates it gives rise to a large number of granules which later collect into chromosome-like masses. However, the relationship is certainly sufficiently close to warrant our placing in the same general category; the solid chromatin nuclei of some Sporozoa and Rhizopoda, the karyosomes of higher protozoan nuclei, and the karyosomes and karyospheres,[1] found in the nuclei of metazoan cells.

"Chromatin nucleoli" being of such universal occurrence in protozoan cells, it is to be expected that some metazoan cells exhibit the same structure. As I have already shown, such examples are fairly common in germ cells and seem to be especially numerous in somatic cells and in the female germinal elements. So far as I know they occur only in cells which are undergoing especially long periods of mitotic inactivity. Such is certainly very evidently true of the germ cells of *Scolopendra heros* where, during the time of their presence, the pseudo-germinal vesicle stage, the cell increases many times in size.

The pseudo-germinal vesicle stage is succeeded by the active prophase of the first maturation division. This phase is inaugurated by modifications in the cytoplasm of the cell and by the migration of the centrosome to the nuclear membrane. Upon reaching this structure the centrosome divides and the two parts begin their divergent courses.

By the time this is well begun the nucleus also commences to show signs of activity. The linin reticulum becomes more ragged and the threads are now composed of finer granules. But the most important phenomena are those to be observed in connection with the karyosphere. At a casual glance this structure seems to have undergone no change, but upon careful examination it is found that its outline is now more irregular and its consistency

[1] In the above terminology I have limited the term karyosome to structures other than chromosomes found within the nucleus which are apparently composed exclusively of chromatin. The karyosphere is much more highly organized, as it contains chromatin (in a granular, reticular or spireme form), karyoplasm, *i. e.*, linin and karyolymph. It is in fact a miniature nucleus.

more spongy (Fig. 9, *e*). This continues to become more marked until in a short time one or several projections may be seen extending from its surface (Figs. 10, 11). These granular filaments stain densely and are similar in all respects to the chromatin segments characteristic of the " spireme " stage. They continue to lengthen until when they have attained a certain size they become detached from the karyosphere and lie free in the nuclear space (Figs. 10, 11, 12). These segments continue to form until they are exactly equal in number to the threads formerly seen in the early spermatocytes.

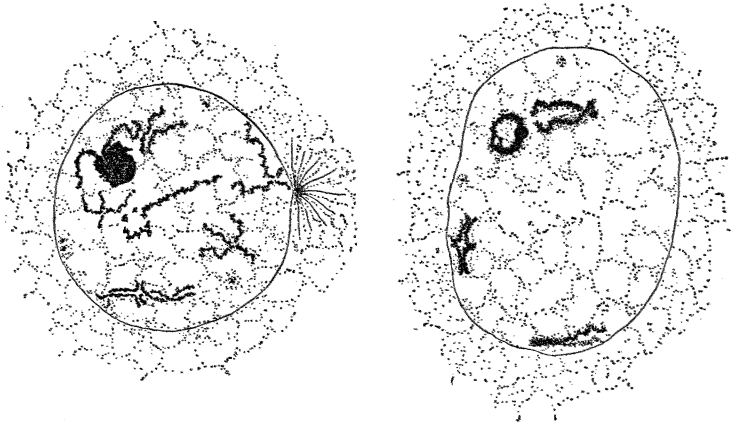


FIG. 11. × 1,440 dia. Nucleus of about the same stage as seen in a thinner section. " Spireme " structure of the karyosphere shown. Tetrads in various stages of formation. One centrosome with rays to be seen upon nuclear membrane, the other not included in the section.

FIG. 12. × 1,440 dia. Later stage, showing the unwinding of the last chromosome from the karyosphere, thus again disclosing the accessory chromosome.

As this process proceeds, the size of the karyosphere decreases proportionately until finally nothing remains except the body with which the transformation started, the accessory chromosome. From this fact alone we might indeed be justified in concluding that the chromosomes are derived from the karyosphere, but no such assumption is necessary. Absolute proof of the truth of this statement is at hand. Actual observations of all the stages incident to chromosome formation may easily be made so that it is impossible for the observer to escape the very evident conclusions to be drawn therefrom. Figs. 10 and 11

are camera lucida drawings of nuclei which show the origin of the chromosomes as well as could be done even by the use of diagrams. In Fig. 10 the karyosphere is very much reduced in size and of an irregular shape; from this three filamentous projections extend, at the distal end of each of which is to be seen a segment evidently only just detached. This is already undergoing the process of tetrad formation. Fig. 12 represents a considerably later stage in which the last chromosome is leaving the karyosphere and the accessory chromosome is again unmistakably to be seen. From these observations I believe no other conclusion can be drawn than that stated above.

To sum up briefly: At the time of the pseudo-germinal vesicle stage, all the chromatin of the cell is aggregated in the karyosphere which consists of a number of fine chromatin segments closely massed about the accessory chromosome. In the succeeding prophase, the first change has to do with the loosening of this mass of filaments. Later several ends become free and by simply uncoiling, give rise to slender processes extending out into the nucleus. These become detached and new threads are protruded until sixteen segments are present, which together with the accessory chromosome make up seventeen, the number of chromatin elements characteristic of the spermatocytes of *Scolopendra*.

Several investigators mentioned before have traced in considerable detail the origin of chromosomes from nucleolus-like bodies. Blochmann, '82 (*Neritina*) says: "Das die Elemente der Kernblatte aus Theilstücken des Nucleolus entstehen, kann bei unserem Objeke keinen Zweifel unterliegen, da ich alle Uebergangszustände vom unversehrten Nucleolus bis zur angebildeten Kernplatte beobachtet habe." In the germ cells of *Mus* a like condition undoubtedly exists according to the investigations of Hermann, '89; Holl, '93; and Sobotta, '95. Hermann reports "chromatin nucleoli" as present in the cells at various stages of spermatogenesis. Holl shows that in the germinal vesicle of the mouse ovum there is a large nucleolus composed chiefly of chromatin from the substance of which the chromosomes of the first maturation mitosis are formed. Sobotta asserts that during fertilization the chromatin of each pronucleus is in the form of

one or several large nucleoli of pure chromatin from which are derived the chromosomes of the succeeding division. In the maturation of the egg of *Distaplia*, Davidhoff, '89, has observed similar phenomena.

C. Schleider, '91, believes that the large nuclei found in the eggs of *Echinodermata* are but reserve masses of chromatin. That this is true under some conditions at least, is shown by the recent experiments of R. Hertwig, '96, and Wilson, '01. Wilson finds that, in one series of eggs chemically fertilized with MgCl solution, the chromosomes functioning in mitosis are obtained by the breaking down of the large densely staining "nucleolus." "Its contour becomes irregular and its texture loose. A little later it assumes a spongy appearance and short irregular processes are extended from its periphery. Enlarging still more it now gives almost the appearance of a close, broken spireme from the ends of which chromatin threads here and there project." These threads later form the chromosomes. As will be readily seen this process in *Toxopneustes* is very similar in many respects to that occuring in *Scolopendra.*

The chromatin segments as they arise from the karyosphere in *Scolopendra* are long, slender, granular filaments usually considerably curved and distorted (Figs. 10, 11, 12). They are arranged irregularly throughout the nuclear area supported by the linin reticulum. Very soon after their detachment from the karyosphere, they are seen to be divided longitudinally along their entire length. Owing to the length and distortion of these segments they frequently assume very fantastic shapes. In some cases the two parts are coiled or twisted about each other like the strands of a rope (Fig. 11) while the two halves of other chromosomes may be separated by a considerable distance (Fig. 10). This cleavage of the segment very evidently represents the longitudinal division of the chromosome, and as the chromosome is first divided in this manner in the prophase it is, I believe, justifiable to conclude with McClung, '00, that the first maturation mitosis accomplishes the equational division of the chromatic elements. Apparently the next change in the structure of the split segments is shown in Fig. 13, *a*. This first becomes apparent as a weakening of the two parts of the segment at about their mid-

dle. The threads show a tendency to bend at a more or less acute angle at this point, and this soon results in a transverse division of each of the parts of the chromosome. Thus each of the chromatin segments has been divided into four parts and may from now on be called a tetrad. Following the terminology suggested by McClung, '00, I shall designate each of the parts going to make up the tetrad or chromosome of the first spermatocyte, a chromatid. By this system I believe much confusion will be prevented.

After the cross division has become established the next change observable is shown in Fig. 13 *b*, *c*, *g*. The chromatids revolve upon each other in such a manner that the ends at the point of transverse cleavage are drawn out parallel to each other and an irregular cross-shaped figure is thus formed (Fig. 13, *d*, *e*).

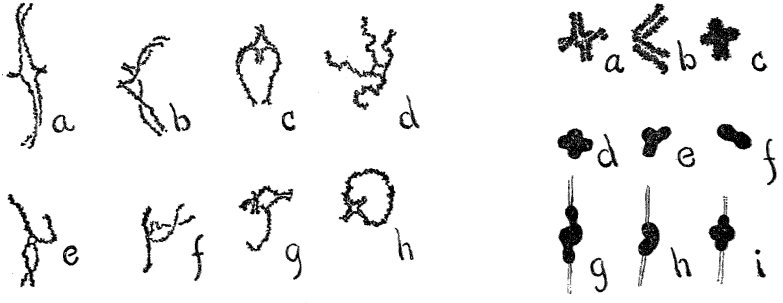



FIG. 13. × 1,400 dia. Various stages and modifications of tetrads. *a*, *b*, *c*, early stages in the process of transverse division. *d*, typical tetrad of mid-prophase. *e*, *f*, *g*, *h*, modifications of the tetrad type.

FIG. 14. × 1,440 dia. Later stages in the history of the tetrad. *a*, typical cruciform tetrad of later prophase. *b*, "double V" form of chromosome at the same stage. *c*, *d*, successively later stages of the cross figure. *e*, *f*, apparent modifications of tetrad in later prophase. *i*, *h*, typical chromosomes at beginning of metaphase. *g*, tetrad undergoing longitudinal division.

This cross-shaped figure is composed of four arms of about equal length each of which is split longitudinally. Owing to the very irregular shape of these arms, the cleavages are masked and are often very hard to demonstrate. However, in later stages when the arms are greatly shortened the bipartite structure is readily seen (Fig. 14, *a*, *b*, *c*), and is also strongly indicated even in the earlier stages by the diamond-shaped opening at the center

of the tetrad. When seen *en face* this opening is always square or diamond-shaped with the angles directed toward the arm, indicating that it is continuous into each arm.

At the stage represented in Fig. 13, *d*, the tetrads are often so distorted that the typical form is lost, but upon studying them more carefully it is seen that they are always referable to the same type. Taking *d* as the type, the more common variations are shown in *b*, *e*, *f*, *g*, *h*. At *b* the formation of the arms, instead of occurring in the plane of the threads, has proceeded in a plane at right angles thereto, resulting in the double V figures first mentioned by Paulmier. At *c*, *h* the long arms of the cross have been curved around and nearly brought in contact. Such distortions observed in later stages of tetrads result in a figure similar in shape to a seal ring, the point of double cleavage representing the seal and the long arms approximating to form an apparently closed circle. Fig. 13, *e*, *f*, *g* are but slight or apparent modifications caused by viewing the tetrads diagonally or in profile.

By later changes the arms of the cross figures are much shortened and the divisions between separate chromatids become very apparent (Fig. 14, *a*, *b*, *c*). However, this shortening and condensation continues and these divisions are entirely obliterated and the chromosome becomes first a granular mass and later an apparently homogeneous one. The tetrads even at this stage vary considerably in shape as shown in Fig. 14, *d*, *e*, *f*. The typical form is represented by Fig. 14, *d*, and by numerous chromosomes in Fig. 15.

During the prophase the tetrads of each nucleus have not developed synchronously, but at any given time are in various stages of formation (Fig. 11). This phenomenon is very easily explained. On account of the dense massing of the chromatin segment in the karyosphere, but a few elements can separate at one time and it very naturally follows that those first escaping from this body exhibit more advanced development than those arising later. At a short time before the dissolution of the nuclear membrane, however, the more tardy individuals have overtaken their fellows and all now appear as homogeneous bodies exhibiting strongly all the chromatin reactions.

As will be seen from the foregoing description, the tetrads occurring in *Scolopendra* are similar to those previously described by other authors in various arthropods. What may be taken as the type of these figures was first reported by Paulmier, '99, in Hemiptera and McClung, '00 and '02, in Orthoptera. Structures differing slightly in detail, the apparent divergence evidently being due more to interpretation than to any essential morphological variations, have been found in other arthropods by Henking, '91 (*Pyrrhocoris*), Vom Rath, '95, '97 (*Gryllotalpa*), Toyama,

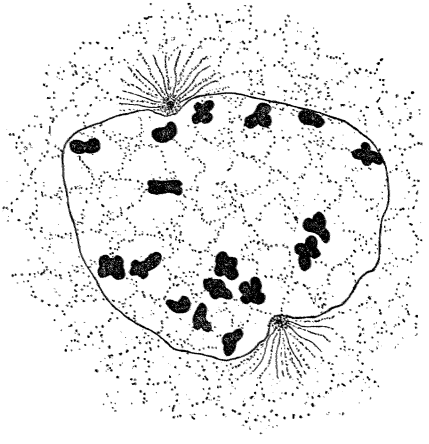


FIG. 15. × 1,440 dia. Nucleus of first spermatocyte during late prophase, showing various modifications in the shape of the chromosomes at this time. The accessory chromosome is seen to be split longitudinally. Centrosomes with well-developed astral rays at opposite poles of the nucleus.

'94 (silkworm), Rückert, '95 (Copepoda), Montgomery,[1] '98, '00, '01 (Hemiptera, *Peripatus*), Blackman, '01 (*Scolopendra*), P. Bouin, '02 (*Lithobius*), Miss Nichols, '02 (*Oniscus*), and others. In the tetrads observed by all of these authors, the cleavages universally represent a longitudinal and a transverse division of the double or bivalent chromosome of the spermatocyte. Apparent discrepancies are undoubtedly due to mere variations in detail or differences in interpretation and denote no real important divergence in the formation of the tetrads.

[1] In his earlier paper Montgomery reported two cross divisions as occurring in *Pentatoma* (*Euchistus*), but in his subsequent publications has denied the accuracy of this observation and now believes that one longitudinal division invariably occurs.

The results of Wilcox, '95, '96 (*Caloptenus*), and de Sinéty, '01 (Orthoptera), however, are indeed radically different. Even here, however, I believe that the divergence is due either to the authors' interpretation of observations, or to insufficient or inferior material. Wilcox asserts that the two spermatocyte mitoses accomplish a double transverse division of the chromosomes. Such is not the case in the western individuals of the same species where a longitudinal, followed by a transverse, division invariably occurs. De Sinéty, working upon the cells of several genera of Orthoptera, asserts that the two divisions are longitudinal. This also appears to be a mistaken conception, as pointed out by McClung, '02. Appearances which upon superficial examination might lead to this view are occasionally met with in Orthopteran material, but when studied closely a different interpretation must always follow. In *Scolopendra* spermatocytes I believe it would be impossible to arrive at this conclusion however strong a preconception the observer may have had. The tetrad figures accompanying this article can by no possibility be logically interpreted as representing anything but a longitudinal and transverse division of the chromosomes. In the interpretation of the first spermatocyte chromosomes and in the sequence of the succeeding divisions I am gratified to note that P. Bouin, working upon other genera of Myriapoda, agrees with my conclusions upon *Scolopendra*.

The tetrad forms which are of most common occurrence in the arthropods are modifications of the cross, double V and ring figures found in *Anasa* (Paulmier, '98) and *Hippiscus* (McClung, '00). It is very probable that all of the other tetrads found in this group are obtained by a greater or less modification of the same process. Such is evidently the case in Copepoda (Ruckert, '94) (Hacker, '95); in *Gryllotalpa* (Vom Rath, '91) and seems also to be true of other invertebrates, *Thalassema* and *Zirphea* (Griffin, '95), *Unio* (Lillie, '95), etc.

The typical arthropod tetrad as exhibited in the Insecta, and in the Myriapoda is obtained in the following manner: The chromatin segments of the matured number as they arise from the spireme stage (Insecta) or from the aggregated segments in the karyosphere (Myriapoda) are long slender threads

of granular chromatin. Each thread very quickly splits longitudinally, thus giving rise to two long slender segments extending parallel to each other. Very shortly after this longitudinal split is made, apparent indications of the second cleavage may be seen. The first indication of this is a bending of the two halves of the segment at their middle point. This extension may be in exactly opposite directions when the resulting tetrad is of the typical cross shape or may occur in such a manner that the two angles are drawn out parallel to each other, in which case the double V figure results. This stage of the two forms of tetrad figures is shown in Fig. 13, *a*, *b*. The bending of the two segments soon results in a transverse cleavage at the angles as indicated in Fig. 13, *a*, *b*, *c*, *g*, *h*. The short processes thus produced elongate at the expense of the length of the quadripartite segment until a cruciform figure is produced, the four arms of which are of about equal length. Each of these arms is traversed by a split extending its entire length and thus producing a diamond-shaped opening in the center of the X figure. Thus it is brought about that the two adjacent halves of contiguous arms are continuous and form one of the four chromatids derived by the double splitting of the chromatin segment (Fig. 14, *a*). The structure of the tetrad is best seen in *Scolopendra* in the later stages of tetrad formation when the arms have shortened and when the chromatin granules are more densely grouped together (Fig. 14, *a*, *b*, *c*). In the late prophase the chromosome becomes homogeneous and assumes the four-lobed shape represented in Fig. 14, *d*, *e*, *f*. The diamond-shaped opening at the center and the splits in the arms are entirely obliterated.

While these fundamental changes have been taking place in the other elements the accessory chromosome has also undergone some alteration. As it emerges from the karyosphere this element is a homogeneous spherical mass of chromatin. (Fig. 12). In the late prophase it is no longer spherical but presents the appearance of a rod the two ends of which are constricted (Fig. 15). This constriction undoubtedly indicates a longitudinal division.

When its history is considered this divergence in form from the tetrads surrounding it is very readily explainable and is pre-

cisely what should be expected. Each of the other chromosomes is derived by the fusion of two of the spermatogonial chromosomes during the telophase of the last mitosis of the division period. On the other hand, the accessory chromosome is descended directly from a single element of the spermatogonium. This being true, it is but logical to expect it to behave differently. The primary object of the spermatocyte period is the reduction of the chromosomes to one half the somatic number. It is usually, if not invariably, the case, in arthropods at least, that this period is characterized by two divisions of the chromosomes, a longitudinal and a cross division. It is generally assumed that, by one of these divisions — the transverse division—reduction is accomplished by the pulling apart of the chromosomes at the point at which they were united in the preceding synapsis. Now as the accessory chromosome is not obtained by the union of two spermatogonial chromosomes, this reducing division is not necessary and does not take place. For these reasons while the ordinary chromosomes are each composed of four parts, *i. e.*, are tetrads, this modified chromosome is made up of but two parts, *i. e.*, is a dyad. Furthermore, it is logically to be expected that the accessory chromosome being dyad in its nature would take part in only one of the succeeding divisions. This peculiarity has indeed been observed by many investigators of insect spermatogenesis and several explanations more or less supported by observed facts, are offered in explanation thereof.

In *Scolopendra*, as in other arthropods, the longitudinal division of the chromosomes occurs in the first spermatocyte mitosis. Strong indications of the character of this cleavage may be seen in the metaphase of the first spermatocyte. Fig. 14, *i* represents a typical chromosome at the time of the formation of the first maturation spindle. At *g* is shown a tetrad of the same kind undergoing metakinesis. By a comparison of these two chromosomes it becomes evident that it is a longitudinal division of the element which occurs. The mantle fibers are attached to the two ends, and when the force which separates the halves of the two chromosomes is applied, the two parts glide over each other and seem to separate with the greatest reluctance. The strongest proof that we are here dealing with an equation division,

however, is to be found in the prophase. As I have already noted the longitudinal split is the first made manifest at that time, hence logically would be expected to preceed the transverse division, which does not appear until later. Further proof of the sequence of the divisions is found in the second spermatocyte where, as will be presently seen, a cross division of the chromosomes certainly occurs.

The question as to the sequence of the two spermatocyte division, while probably of not any vital importance, has been the subject of considerable controversy. By far the greater number, however, agree that the equation division comes first, and is succeeded by the reduction division. Ruckert, '92, Hacker, '92, McClung, '00, '02, Blackman, '01, P. Bouin, '02, in arthropods, and Bolles Lee, '97, Linville, '00, Griffin, '99, Klinckowström, '97, Francotte, '97, and Van der Stricht, '98, in other invertebrates, have arrived at this conclusion. While the opposing view — *i. e.*, that the reduction division precedes — is held by Vom Rath, '92, '95, Henking, '90, Paulmier, '99, and Montgomery, '98, '00, '01, in anthropods and Lillie, '01, in molluscs. In arriving at this latter conclusion the criterion invariably used is the appearance and behavior of the elements during the two mitoses. But during the metaphase the chromosomes are always so compact that the cleavages shown in the prophase are entirely obliterated, and the manner of division therefore cannot be determined with certainty. An example of the likelihood of misinterpretation of the nature of these divisions is shown by Griffin, '99, *Thalassema*. Here the first division is very evidently longitudinal, and upon superficial observation the second also appears to be of the same nature. But when the phenomena observed in the prophase are considered, it is evident that this cannot be true, as an indubitable transverse cleavage was to be seen at that time. Upon further study Griffin shows his first impression to be false, for the second division is in reality a reducing division.

In all of the investigations with which I am acquainted it has been reported that the longitudinal cleavage is the first to be made evident in the prophase. Then I believe it is but logical to conclude that this division is completed by the first spermatocyte mitosis, especially when this has been shown to be the case in a

great number of cells. Of course it is possible that the process varies in different animals, but it is not probable, for if the sequence of the actual divisions varies, we should naturally expect the prophase phenomena to vary in a like manner. No such variation seems to exist.

The chromosomes as they occur in the metaphase are arranged in no definite equatorial plate but are scattered irregularly throughout the equatorial region of the spindle (Fig. 16). It is also noticeable that the chromosomes do not divide synchronously.

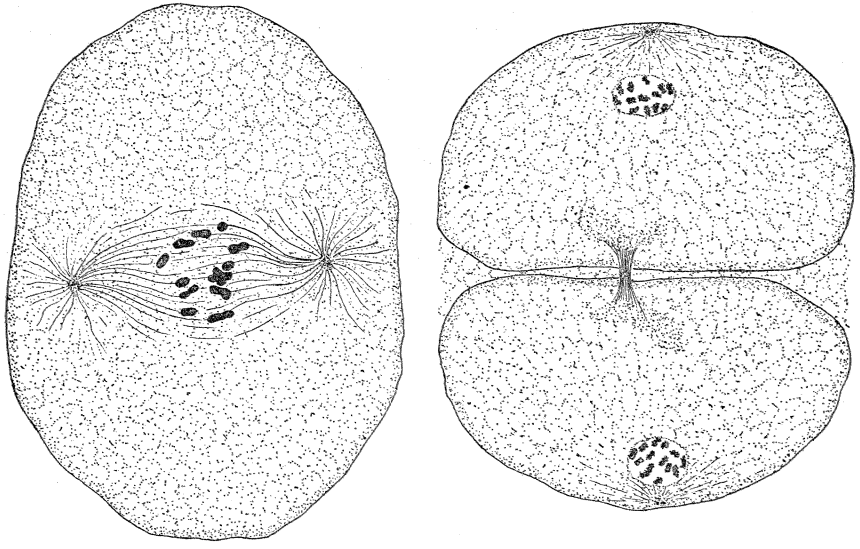


FIG. 16. × 960 dia. Early metaphase of first spermatocyte. Showing the diversity in shape of the chromosomes, and their irregular arrangement in the equatorial region.

FIG. 17. × 960 dia. Telophase of first spermatocyte, showing the unequal division of the chromatin, the accessory chromosome being present in one cell while it is absent in the other.

While some still plainly show their tetrad character, others have completed their separation and have already started toward the poles.

Owing to the approximately equal size of all the chromosomes and the diversity of shapes which they present it has not been found possible to trace the history of the accessory chromosome during the first metakinesis. However, from an examination of the telophase succeeding, it becomes evident that this element

in *Scolopendra* undergoes processes analogous to those reported in insects by a number of investigators. It is found in one of the cells resulting from the first mitosis and does not occur in the other (Fig. 17) showing that it takes no part in this division but goes over to one cell undivided.

With the reconstruction of the daughter nuclei, all of the chromosomes except the accessory become granular (Fig. 18) and present the appearance of rather short rods of diffuse chromatin, the center of each of which is slightly constricted, thus

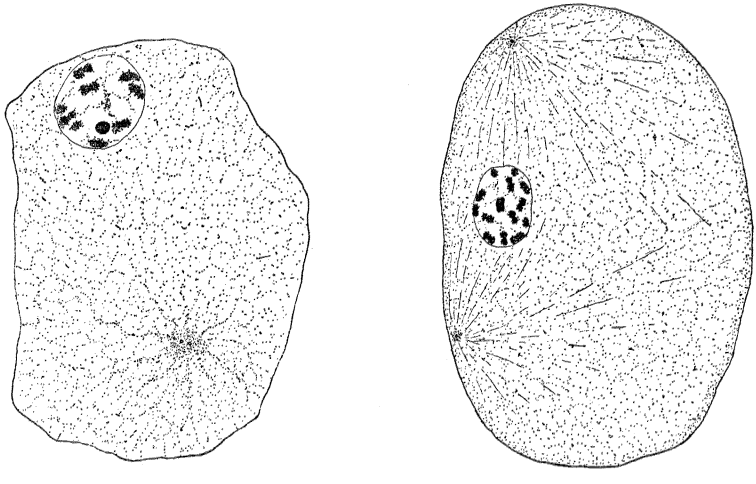


FIG. 18. × 960 dia. Prophase of a second spermatocyte containing the accessory chromosome. The ordinary chromosomes are diffuse and of a dumb-bell form, while the accessory is homogeneous and spherical. Centrosome and persisting archoplasm visible.

FIG. 19. × 960 dia. Late prophase of second spermatocyte. Chromosomes are less diffuse. Accessory chromosome seen to be constricted longitudinally, while the others show indications of a transverse division.

producing a dumb-bell-shaped body. In the succeeding stages these become more dense (Fig. 19) and finally go to the equatorial plate as small homogeneous bodies of a distinctly lobate structure. When arranged in the equatorial region (as in the first division, there is no true equatorial plate) the lobes of these bodies are directed toward the poles of the spindle, thus giving further basis for the conclusion that we have here a cross division of the chromosome.

During the metaphase, however, one of the chromatic elements does not show the dumb-bell-shape characteristic of the rest, but is very evidently a rod split in the opposite direction, *i. e.*, longitudinally. This peculiarity is also to be seen in the preceding prophase where the accessory chromosome is of the same shape as in the first spermatocyte prophase. As it is seen during the early metaphase, this element is arranged with the plane of cleavage at right angles to the spindle (Fig. 20), but upon the contraction of the mantle fibers which are attached to

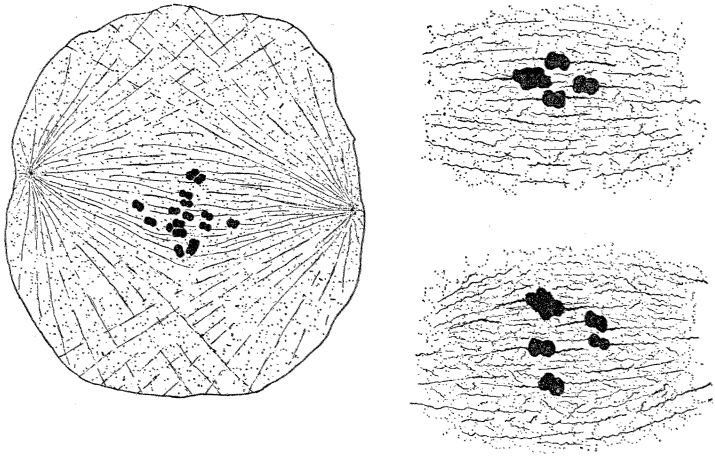


FIG. 20. × 960 dia. Metaphase of second spermatocyte. The difference in shape and orientation existing between the accessory and the other chromosomes is evident.

FIG. 21. × 1,920 dia. High magnification of same stage, showing the differences exhibited by the accessory chromosome in the relation of the chromatids and in the attachment of the mantle fibers.

FIG. 22. × 1,920 dia. Slightly later stage; showing the effect of the contraction of the mantle fibers on the orientation of the accessory chromosome.

opposite ends of the element it revolves through an arc of 90° (Figs. 21, 22) and the component chromatids as they are pulled apart seem to glide over each other (Fig. 22) in a manner similar to that already noted as characteristic of the ordinary chromosomes during the first mitosis.

It will be seen by consulting the accompanying figures that the behavior of the other elements is quite different. These are arranged with their long axis parallel to that of the spindle, the

separation of the chromatid occurring along the equatorial plane at the place of constriction. This very evidently represents a cross division of the chromosome.

In the division figures of one half of the second spermatocytes, all the chromosomes are of one type (the dumb-bell form), the accessory chromosome not being present. Thus it will be readily seen that the cells arising from two spermatocyte mitoses are divided into two classes of equal numbers — those which possess the accessory and those which do not. Similar phenomena have been observed in the cells of a number of insects by Henking, '90, Paulmier, '99, McClung, '00, '02, de Sinéty, '01, and others.

Regarding the function of the modified chromosome, two theories have now been advanced. Paulmier in his paper on *Anasa* puts forth the theory that the "small chromosome represents characteristics which are being eliminated from the race." He bases this conclusion entirely upon the failure of the element to divide in one spermatocyte division. Montgomery in his later papers adopts the conclusions of Paulmier and believes with him that it is a chromosome undergoing the process of elimination.

McClung, '02, however, in a paper in which he considers in detail all of the reported observations upon the accessory chromosome, formulates an hypothesis which ascribes a very different function to this element. He maintains that the mere fact of the unequal apportionment to the spermatozoa would not necessarily indicate that the element is degenerating, and in addition there are other facts which militate strongly against such a conclusion. The extreme nicety with which this element is excluded from contact with the others in most stages, especially in the spermatogium, would seem to indicate a very different and much greater significance. This exclusiveness, taken in connection with the fact that exactly one half the spermatozoa contain the accessory chromosome, suggests the theory that it has to do with the determination of sex, as this is the only respect in which the progeny are divided into two classes of equal numbers. Although no positive proof is advanced to support this theory the author establishes in a very logical manner the probability of the accessory chromosome representing such a function. It seems to

possess all the characteristics required of such an element. My observations upon *Scolopendra* surely lend support to this theory. Definite proof of the function of this structure can only be obtained, however, by a study of the process occurring in the fertilization and cleavage of the egg.

My observations upon the accessory chromosome in *Scolopendra* have added very little to our knowledge of this element, except in so far as they help to show its wide distribution and the great similarity of its behavior in widely separated groups. Indeed in all important particulars the phenomena accompanying the development of this structure are identical in Chilopoda and Orthoptera, although the minor details of the process vary considerably. In both groups the element is derived directly from a single spermatogonial chromosome, and for this reason takes no active part in the phenomena of synapsis. During the prophase when the other chromosomes divide into four chromatids and form tetrads, this element, as would be expected from its origin, cleaves but once and that longitudinally. In the two succeeding divisions it is divided but once and thus is present in but one half of the spermatids. The differences, although at times puzzling, are in reality slight and unimportant. Thus, at the time when all of the chromatin is aggregated in the karyosphere, the accessory chromosome cannot be distinguished except in the most favorable cases; but from the study of these thin, well-differentiated sections we are justified in saying that even in the pseudo-germinal vesicle stage this element retains all its ordinary characteristics. In the mataphase of the first spermatocyte it cannot be distinguished from the other chromosomes as it can in Orthopteran material, because it is of approximately the same size as these. In the second maturation division, however, it is again very evident, by reason of the fact that it divides longitudinally while the other chromosomes divide transversely.

These variations, as has been said, are unimportant modifications of behavior and do not represent such fundamental differences as seem to exist between the "small chromosome" (Paulmier) or the "chromatin nucleolus" (Montgomery) in Hemiptera and the accessory chromosome in Orthoptera. If the observations of Paulmier and Montgomery concerning the origin of this element are

correct, it is indeed doubtful whether the bodies described represent the same structure as the accessory chromosome. The chromosome $x$ of *Protenor* (Montgomery, '01) would seem more closely to approach this modified element in origin and behavior.

I am glad of this opportunity of expressing my gratitude to Dr. C. E. McClung for valuable advise and criticism throughout the progress of this work.

LABORATORY OF ZOÖLOGY AND HISTOLOGY,
UNIVERSITY OF KANSAS, April 11, 1903.

## BIBLIOGRAPHY.

**Blackman, M. W.**

'01 The Spermatogenesis of the Myriapods, I. Notes on the Spermatocytes and Spermatids of Scolopendra. Kans. Univ. Quart., X., 1901.

**Blochmann, F.**

'82 Ueber die Entwicklung der Neritina fluviatilis. Zeit. f. Wiss. Zool., 36., 1882.

**Bouin, P.**

'01 Sur le fuseau, le residu fusorial et le Corpuscule intermediare das les cellules seminales de Lithobius. forficatus. C. R., 1901.

**Bouin, P.**

'02 Reduction chromatique chez les Myriapods comp. rend. de l'assoc. des anat.

**Calkins, G. N.**

'98 The Phylogenetic Significance of Certain Protozoan Nuclei. Ann. N. Y. Acad. Sci., 11, 1898.

'98 Mitosis in Noctiluca. Journ. Morph., 15, 3, 1898.

'01 The Protozoa. Colum. Univ. Biol. Series. Macmillan, New York, 1901.

**Carnoy, J. B.**

'85 La Cytodiérèse chez les arthropodes. La Cellule, 1, 1885.

**Carnoy, J. B., & Lebrun, H.**

'97 La vésicule germinative et les globules polaires chez les Batrachiens. La Cellule, 12, 1897.

'98 La Vésicle germinative et les globules polaires chez les Batrachiens. La Cellule, 14, 1898.

'00 La vésicule germinative et les globules polaires chez les Batrachiens. La Cellule, 16, 1900.

**Eisen, G.**

'00 The Spermatogenesis of Batrachoseps. Journ. Morph., 17, 1900.

**Fick, R.**

'93 Ueber die Reifung und Befruchtung des Axolotleies. Zeit. f. Wiss. Zool., 56, 1893.

**Griffin, B. B.**

'99 Studies on the Maturation, Fertilization and Cleavage of Thalassema and Zirphæa. Journ. Morph., 15, 1899.

**Hacker, V.**

'92 Die Eibildung bei Cyclops und Canthocampus. Spengel's Zool. Jahrb., 5, 1892.

**Henking, H.**

'90 Untersuchungen über die ersten Entwicklungsvorgänge in den Eieren der Insecten. 2. Ueber Spermatogenese und deren Beziehung zur Eientwicklung bei Pyrrhocoris apterus, M. Zeit. f. Wiss. Zool., 51, 1890.

**Hermann, F.**

'89 Die postfötale Histogenese des Hodens der Maus bis zur Pubertät. Arch. f. mikr. Anat., 50, 1889.

**Hertwig, R.**

'84 Ueber die Kerntheilung bei Actinosphærium Eichhornii. Zeit. f. Natur. w. Jena, 17, 1884.

'96 Ueber die Entwicklung des unbefruchteten Seeigeleies. Festsch. für Gegenbaur, 1896.

**Holl, M.**

'99 Ueber die Reifung der Eizelle bei den Säugethieren. Sitzb. d. Akad. Wissensch. von Wien., 1899.

**Jordan, E. O.**

'93 The Habits and Development of the Newt. Journ. Morph., 8, 1893.

**Lillie, F. R.**

'01 The Organization of the Egg of Unio, Based on a Study of its Maturation, Fertilization and Cleavage. Journ. Morph., 17, 1901.

**Linville, Henry R.**

'00 Maturation and Fertilization in Pulmonate Gasteropods. Bull. Mus. Comp. Zoöl., Harvard Coll., 35, 1900.

**Macallum, A. B.**

'95 On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells. Quart. Journ. Micr. Soc., N. S., 38, 1895.

**McClung, C. E.**

'99 A Peculiar Nuclear Element in the Male Reproductive Cells of Insects Zoöl. Bull., 2, 1899.

'00 The Spermatocyte Divisions of the Acrididæ. Kans. Univ. Quart., 9, 1900.

'02 The Accessory Chromosome — Sex Determinant? Biol. Bull., 3, 1902.

'02 The Spermatocyte Divisions of the Locustidæ. Kans. Univ. Sci. Bull., 1, 1902.

**Montgomery, T. H., Jr.**

'98 The Spermatogenesis in Pentatoma up to the formation of the Spermatid. Zoöl. Jahrb., 12, 1898.

'98 Chromatin Reduction in the Hemiptera, A Correction. Zoöl. Anz., 22, 1898.

'98 Comparative Cytological Studies, with Especial Regard to the Morphology of the Nucleolus. Journ. Morph., XV., 1898.

'00 The Spermatogenesis of Peripatus balfouri up to the Formation of the Spermatid. Zoöl. Jahrb., 14, 1900.

'01 A Study of the Germ Cells of Metazoa. Trans. Amer. Phil. Soc., 20, 1901.

**Moore, J. E. S.**

'95 On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs. Quart. Journ. Micr. Soc., N. S., 38, 1895.

**Paulmier, F. C.**

'98 Chromatin Reduction in the Hemiptera. Anat. Anz., 14, 1898.

'98 The Spermatogenesis of Anasa tristis. Journ. Morph., 15, 1898.

**Platner, G.**

'86 Die Karyokinese bei den Lepidopteran als Grundlage für eine Theorie der Zelltheilung. Internat. Monatsschr., Anat. Hist., 4, 1886.

**Rath, O. Vom**

'92 Zur Kenntnis der Spermatogenese von Gryllotalpa vulgaris, Latr. Arch. f. Mikr. Anat., 40, 1892.

'95 Neue Beiträge zur Frage der Chromatinreduction in der Samen und Eireife, 1895.

**Rhumbler, L.**

'93 Ueber Entstehung und Bedeutung der in den Kernen vieler Protozoen und in Keimbläschen vom Metazoen vorkommenden Binnenkörper. Zeit. f. Wiss. Zoöl., 61, 1893.

**Ruckert, J.**

'92 Zur Eireifung bei Copepoden. Merkel and Bonnet's Anat. Hefte, 1892.

**Schultze, O.**

'87 Untersuchungen über die Reifung und Befruchtung des Amphibieneies. Zeit. f. wiss. Zoöl., 45, 1887.

**Sinéty, R. de.**

'01 Recherches sur la biologie et l'anatomie des Phasmes. La Cellule, 19, 1901.

**Sobotta, J.**

'95 Die Befruchtung und Furchung des Eies der Maus. Arch. f. Mikr. Anat., 45, 1895.

**Stuhlmann, Fr.**

'86 Die Reifung des Arthropodeneies. Ber. d. Naturforsch. Gesell. v. Freiberg, i. 8, 1886.

**Sutton, W. S.**

'00 The Spermatogonial Divisions of Brachystola magna. Kans. Univ. Quart., 9, 1900.

'02 On the Morphology of the Chromosome Group in Brachystola magna. Biol. Bull., 4, 1902.

**Wallace, Louise.**

'00 The Accessory Chromosome in the Spider. Anat. Anz., 18, 1900.

**Wheeler, W. M.**

'97 The Maturation, Fecundation and Early Cleavage in Myzostoma glabrum. Arch. de Biol., 15, 1897.

**Wilcox, E. V.**

'95 Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. Bull. Mus. Comp. Zoöl., Harvard Univ., 27, 1895.

'96 Further Studies on the Spermatogenesis of Caloptenus femur-rubrum. Bull. Mus. Comp. Zoöl., Harvard Univ., p. 29, 1896.

**Wilson, E. B.**

'01 Experimental Studies in Cytology. I. A Cytological Study of Artificial Parthenogenesis in Sea Urchin Eggs. Archiv f. Entwm., 12, 1901.

'01 Experimental Studies in Cytology. II. Some Phenomena of Fertilization and Cell Division in Etherized Eggs, 1901.

III. The Effect on Cleavage of Artificial Obliteration of the First Cleavage Furrow. Archiv f. Entwm., 14, 1901.